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Alterations in the production and concentration of selected alkaloids as a function of rising atmospheric carbon dioxide and air temperature: implications for ethno-pharmacology

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Abstract

The influence of recent and projected changes in atmospheric carbon dioxide concentration [CO_2] with and without concurrent increases in air temperature was determined with respect to growth characteristics and production of secondary compounds (alkaloids) in tobacco (*Nicotiana tabacum* L.) and jimson weed (*Datura stramonium* L.) over a ca. 50-day period. Rising [CO_2] above that present at the beginning of the 20th century resulted in consistent, significant increases in leaf area, and above ground dry weight (both species), but decreased leaf area ratio (LAR) and specific leaf area (SLA) in jimson weed. Increased temperature resulted in earlier development and increased leaf area for both species, but increases in above ground final dry weight were observed only for jimson weed. The secondary compounds evaluated included the alkaloids, nicotine, atropine and scopolamine. These compounds are generally recognized as having impacts with respect to herbivory as well as human physiology. Rising [CO_2] reduced the concentration of nicotine in tobacco; but had no effect on atropine, and increased the concentration of scopolamine in jimson weed. However, because of the stimulatory effect of [CO_2] on growth, the amount of all three secondary compounds increased on a per plant basis in both species. Temperature *per se* had no effect on nicotine or scopolamine concentration, but significantly increased the concentration and amounts of atropine per plant. Overall, the underlying mechanism of CO_2 induced changes in secondary compounds remains unclear; however, these data suggest that the increase in [CO_2] and temperature associated with global climate change may have significant effects not only with respect to herbivory, but on the production of secondary compounds of pharmacological impact.

Keywords: atropine, jimson weed, nicotine, scopolamine, tobacco

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Introduction

Secondary plant metabolites are generally recognized as being critical to plant protection because they serve as a chemical defense against herbivores or invasion by pathogens. Any alteration in the abiotic environment that affects their production or concentration is likely to have ecological or economic consequences.

At present, it is generally recognized that two aspects of the abiotic environment undergoing anthropogenic change are atmospheric carbon dioxide concentration and air temperature. Data from the carbon dioxide information analysis center (CDIAC) indicate a $\sim 30\%$ increase in the global background concentration of atmospheric carbon dioxide [CO_2] from 290 to 375 μatm during the last 100 years, with the largest increase in recent decades (i.e. 310–375 μatm since 1955, Keeling & Whorf, 2001). It is anticipated that the global background [CO_2] will continue to increase with concentrations projected to approach 500–700 μatm by the end of the current century [Intergovernmental Panel on Climate Change (IPCC) scenario's IS 92e and IS 92 a, respectively in Schimel *et al*. (1996)]. The observed

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change in atmospheric [CO_2] has been accompanied by documented increases in other radiation trapping gases such as methane (CH_4) (0.9% increase per year), nitrous oxide (N_2O) (0.25% per year), and chlorofluorocarbons (CFCs) (4% per year). Recent evaluations by the IPCC (www.ipcc.ch/pub/reports.htm) based, in part, on an assessment by the US National Academy of Sciences, have indicated that the rise of [CO_2] and associated 'greenhouse' gases could lead to a 3–12 °C increase in global surface temperatures during this century.

To date, a number of investigations have examined the role of either increasing CO_2 or temperature on secondary compounds including isoprene, terpenes, tannin, flavonol and/or phenolic production (e.g. Kainulainen *et al*., 1998; Heyworth *et al*., 1998; Gebauer *et al*., 1998; Lavola *et al*., 2000; Singsaas & Sharkey, 2000; Zavala & Ravetta, 2001). Such studies have been undertaken in part, as a means to better understand plant thermal tolerance, or changes in herbivory in response to global climate change.

While the physiological function of these metabolites regarding plant protection is well recognized, many of these secondary compounds are also acknowledged as having pharmaceutical value. For example, in developed countries, 25% of all prescriptions dispensed from community pharmacies from 1959 through 1980 contained plant extracts or active principles prepared from higher plants (e.g. codeine, Farnsworth *et al*., 1985). For developing countries however, the World Health Organization (WHO) recently reported that more than 3.5 billion people rely on plants as components of their primary health care (WHO, 2002).

In spite of the importance of botanically derived secondary compounds in human health, surprisingly few studies have examined how the increase in [CO_2] and/or temperature associated with climate change is likely to affect their production. Exceptions include an increase in the antidepressants, hypericin and pseudohypericin in St Johns wort (*Hypericum perforatum* L.), and the cardiac glycoside, digoxin, in wooly foxglove (*Digitalis lanata* Ehrh.) at a [CO_2] of 1000 μatm relative to current ambient concentrations (Stuhlfauth & Fock, 1990; Zobayed & Saxena, 2004); as well as increases in purported anticancer constituents in spider lily (*Hymenocallis littoralis* (Jacq.) Salisb.) at 700 μatm relative to ambient (Idso *et al*., 2000). However, no data are available regarding metabolite changes related to the increase in atmospheric [CO_2] that has already occurred, nor to concurrent changes in [CO_2] and temperature for secondary compounds, including alkaloids, whose uses as pharmaceuticals in human systems are recognized globally in ethnopharmacology (e.g. Schultes & Reis, 1995).

To address these uncertainties, we initiated an assessment of the impact of climatic change on three alkaloids: nicotine, atropine and scopoalamine. The toxicological and pharmaceutical applications of these secondary plant chemicals are universally acknowledged as having significant negative (e.g. nicotine) and positive (e.g. atropine) impacts on human health (Topliss *et al*., 2002). Overall, the goal of the current investigation was to evaluate whether recent or projected changes in [CO_2] and/or temperature (i.e. those associated with global climate change) altered the concentration and whole plant production of these compounds using tobacco (an internationally recognized delivery system for nicotine), and jimson weed (used extensively in ethno-pharmacology as a means to deliver atropine and scopolamine), as model systems. Plants of both species were grown for approximately 50 days after sowing (DAS), the time of maximum vegetative growth and subsequent concentration of secondary chemicals associated with herbivory (i.e. concentration is highest in younger, sensitive plant material, e.g., Schultes & Reis, 1995; Ohnmeiss & Baldwin, 2000). A secondary objective was to evaluate concomitant CO_2 and/or temperature -induced changes on the initial growth and phenology of these same species.

Materials and methods

Experimental conditions

Because no system has yet been devised to expose plants to subambient [CO_2] under field conditions for 24 h day^{-1} (see Mayeux *et al*., 1993) these experiments were conducted in controlled environment chambers. Seed from cultivated tobacco (*Nicotiana tabacum*, cv 'Petit') and seed from local populations of jimson weed (*Datura stramonium*) were grown using three controlled environmental chambers (EGC Corp., Chagrin Falls, OH, USA) with each chamber maintained at one of three carbon dioxide concentration set-points, 280, 370 and 720 μmol mol^{-1} for 24 h day^{-1}. These concentrations approximated the [CO_2] present at the beginning of the 20th century, the current concentration, and that projected for the end of the 21st century. Actual [CO_2] values ($\pm$ SD) averaged throughout the experiment were 294 $\pm$ 22, 378 $\pm$ 18 and 690 $\pm$ 26 μmol mol^{-1}. Two to three seeds of each species were sown separately in 0.6 L pots filled with vermiculite for jimson weed, promix for tobacco, and thinned to one seedling 4–6 days after emergence. For each [CO_2], 35–40 plants of each species were grown. All pots were watered to the drip point daily with a complete nutrient solution containing 14.5 mmol m^{-3} nitrogen.

For each chamber, two different sets of temperatures were used. These varied in a diurnal fashion from an

overnight low of either 15 or 20 °C to a maximum afternoon value of either 30 or 35 °C, with average daily (24 h) values of 22.1 or 27.1 °C, respectively. Average daily values for the Virginia/North Carolina region from May through September are 22.3, so the 22.1 and 27.1 values were considered to represent ambient and ambient + 5 °C. An increase in temperature of 5 °C, is consistent with most short-term (~ 50 year) global change scenarios regarding air temperature (McCarthy *et al*., 2001). Light (photosynthetically active radiation, PAR) was also altered diurnally in conjunction with temperature with the highest PAR value (~ 1000 μmol $m^{-2} s^{-1}$) occurring during the afternoon. Daily PAR was 14 h, supplied by a mixture of high-pressure sodium and metal halide lamps and averaged 26.5 mol $m^{-2} day^{-1}$ for all chambers. The CO_2 concentration of the air was controlled by adding either CO_2 or CO_2-free air to maintain the desired CO_2 concentration. CO_2-free air was obtained using a Ballston 75–60 type CO_2 scrubber (Ballston Filter products, Lexington, MA, USA). Injection of CO_2 and CO_2-free air was controlled by a TC-2 controller using input from an absolute infrared gas analyzer (WMA-2, PP Systems, Haverhill, MA, USA). Temperature, humidity and [CO_2] were recorded every 15 min and averages recorded on a daily basis for all experimental runs. Additional details concerning the operating system can be found in Ziska *et al*. (2001).

Vegetative harvests

Destructive harvest of five to six plants for each [CO_2] and temperature occurred at approximately weekly intervals beginning at 16 and 28 DAS for jimson weed and tobacco, respectively. For all harvests, leaf area was determined photometrically using a leaf area meter (Model 3100, Li-Cor Corporation, Lincoln, NE, USA). In addition to leaf area, dry mass was determined separately for all leaves, stems and floral spikes (jimson weed) for each harvest for all treatments following drying at 65 °C for a minimum of 48 h or until dry mass was constant.

Atropine and scopolamine analysis

After harvesting, all replicated leaf samples of jimson weed were oven dried at 40 °C for an additional 48 h to ensure dryness. Extraction and analysis of all samples were as described by Johnson & Emche (1994), except for the following modifications. A 100 mg (dry wt) from each leaf was individually ground and extracted in a Pierce Reacti-Therm III heating/stirring module (Pierce Biotechnology Inc., Rockford, IL, USA) with 95% EtOH and a conical stir bar. Samples were boiled for 25 min at 85 °C, allowed to cool, the solute was then removed and the pellet reextracted. The extracts were combined and roto-evaporated to dryness at 40 °C. Samples were then redissolved in 2 mL HPLC grade MeOH, filtered through a 0.2 μm syringe filter and stored in sealed vials at 4 °C until GC-MS analysis.

Gas chromatography was performed on a Hewlett-Packard 6890A GC, including an HP 7673A autosample injector and Chemstation software (Hewlett-Packard, Avondale, PA, USA). GC conditions were as follows: detection: MS; detector heater: 300 °C; scanning mass range: 50–550 mass units at 1.61 scans s^{-1}; carrier gas; UHP He; column flow rate 1.2 mL min^{-1} column:DB-5 (5% Phenyl), 30 m × 25 mm i.d., 0.25 μm film thickness (Phenomenex, Torrance, CA, USA); injection temperature: 275 °C; programmable oven temperature: 50–300 °C in increments of 30 °C min^{-1}; run time 11.33 min; injection volume 2 μL in splitless mode. Four point standard curves of atropine and scopolamine (Sigma Chemical Co., St Louis, MO, USA) were used for quantification. All samples were injected in triplicate. Confirmation of the standards and plant alkaloid extracts were made by GC-MS spectral analysis.

Nicotine Analysis

Prior to each destructive harvest, five leaf discs (8.25 cm^2 leaf area) were removed from the most recent, fully expanded leaves of tobacco for all experimental treatments. Leaf discs were harvested between 4 and 6 h after the start of the photoperiod, rapidly transferred to labeled envelopes and immersed in liquid N_2 to stop metabolism. Samples were then lyophilized for 72 h and stored in sealed plastic bags at −20 °C until use.

Freeze-dried tobacco leaf tissue was ground to a liquid N_2 powder in a mortar and pestle and then 10 mg DW was transferred to ground glass tissue homogenizers. Samples were extracted at 4 °C with 1 mL of ice-cold 80% methanol and the homogenates were transferred to 2 mL plastic microfuge tubes and stored on ice. Samples were spun in a model 5415 C microcentrifuge (Brinkmann Instr., Westbury, NY, USA) at 14 000 *g* for 5 min. The pellets were washed with an additional 0.5 mL of 80% methanol and then were microfuged as described above. The supernatants were combined and passed through a 13 mm Millex-HN syringe filter (Millipore Corp., Bedford, MA, USA) containing a 0.45 m nylon filter. The filtered samples were extremely stable and could be stored in sealed cryovials at −20 °C for up to 3 months prior to analysis.

Nicotine concentrations were determined by reversed phase high-pressure liquid chromatography according to Papadoyannis *et al*. (2002). Samples were chromato-

graphed on a $250 \times 4.6\,mm^2$ Alltima C8 (5 μm) analytical column from Alltech Assocs. (Deerfield, IL, USA). The isocratic mobile phase consisted of methanol and 50 mM NH_4^+-acetate (60 : 40) and the flow rate was $1\,mL\,min^{-1}$. Absorbance because of nicotine was measured at 262 nm with a Waters model 490E Multiwavelength Detector (Milford, MA, USA) and injections were with a 20 μL sample loop. For quantitation, standard curves were prepared with known amounts of nicotine (N 0267, Sigma-Aldrich, St Louis, MO, USA) diluted in methanol. Standard recoveries were greater than 90%. Using acidified methanol or a brief heating step did not improve the yield of nicotine from tobacco leaf tissue.

Data analysis

Because only three chambers were available, a complete block design was utilized with runs over time as replications (blocks), with each run consisting of all three CO_2 treatments at each of two growth temperatures. At the end of a given run, CO_2 treatments were randomly assigned to a given chamber and the entire experiment was repeated at ambient and ambient $+5\,^{\circ}C$ growth temperature. Overall, all three CO_2 treatments and both temperature treatments were independently replicated three times. Growth, vegetative and reproductive characteristics as well as nicotine, atropine and scopolamine concentration and plant content were determined using a two-way ANOVA with $[CO_2]$ and temperature as the classification variables (Statview, Cary, NC, USA) for each species at a given sampling date. Treatment comparisons were made using a Fisher's protected least significant difference. Unless otherwise mentioned, differences for any measured parameter were determined as being significant at the $P < 0.05$ level.

Results

Following the initial harvest at 28 DAS, $[CO_2]$ had a significant effect on total above ground biomass for all sampling dates in tobacco (Fig. 1). For leaf area, stem and leaf weight, significant effects of $[CO_2]$ values above 294 μatm were observed when averaged over both growth temperatures. No effect of $[CO_2]$ was observed for leaf area ratio (LAR, an index of resource allocation to leaf production), or specific leaf area (SLA, an index of leaf thickness). Increased air temperature had a significant effect on initial changes in total above ground biomass in tobacco; however, no effect was observed when averaged over all $[CO_2]$ treatments after 50 DAS (Table 1). Although no significant interaction between air temperature and $[CO_2]$ was observed (Table 1), if the highest $[CO_2]$ concentration is excluded,

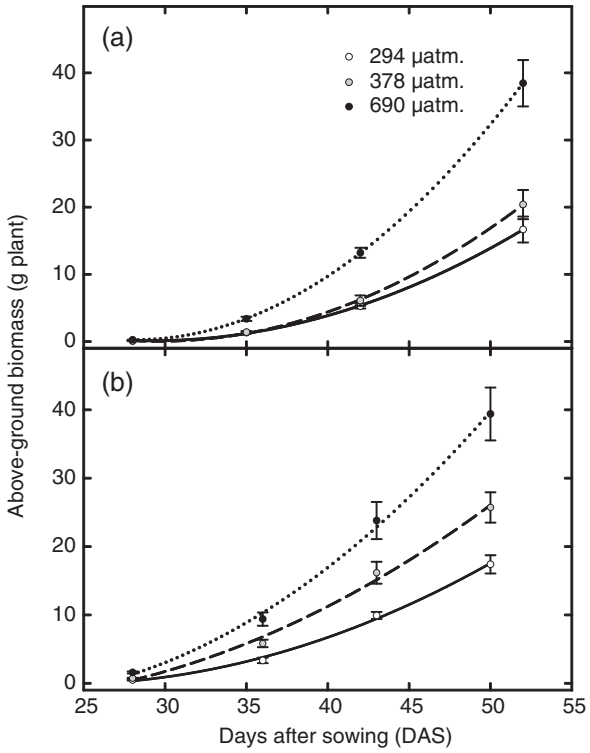

Fig. 1 Total above-ground biomass of tobacco (*Nicotiana tobaccum*, g per plant) over time (days after sowing, DAS) at different $[CO_2]$ (294, 378 and 690 μatm) and air temperature (a and b, 22.1, 27.1 °C, respectively). Significant $[CO_2]$ differences were observed after 27 DAS. Bars are ± SE.

a significant interaction between $[CO_2]$ of 294 and 378 μatm at the higher air temperature is observed (Fig. 1).

For jimson weed, a similar stimulation of above ground biomass was observed for $[CO_2]$ (Table 1, Fig. 2), with significant increases observed in leaf and stem weight ($P = 0.08$ for leaf area). Increased $[CO_2]$ also significantly reduced LAR and SLA (Table 1). Air temperature, in turn, had significant effects on leaf area and unlike tobacco, total above ground dry weight at the end of the experiment. As with tobacco, a significant interaction between $[CO_2]$s of 294 and 378 μatm at the higher air temperature is observed (Fig. 2). Unlike tobacco however, jimson weed flowering occurred early in vegetative growth, with significant increases in reproductive biomass (petals, peduncles and seed) observed in response to both $[CO_2]$ and temperature (Table 1, Fig. 3).

Tobacco leaves are an acknowledged source of nicotine. Overall, $[CO_2]$ reduced the concentration of nicotine, although no effect of growth temperature *per se* was observed (Table 2, Fig. 4). While $[CO_2]$ reduced the concentration of nicotine within the leaves, a significant $[CO_2] \times$ temperature interaction for concentration was also noted (Table 2). Although concentra-

Table 1 Averages and statistical *P* values of the two-way analysis of variance for CO_2 concentration (μatm) and air temperature (°C) effects on vegetative (tobacco, jimson weed) and reproductive characteristics (jimsonweed) at 50–52 and 47 DAS, respectively

	Averages					*P*-values		
Variable	294	378	690	22.1	27.1	CO_2 effect	Temperature effect	$CO_2 \times T$
Tobacco								
Leaf area (m^2)	0.275	0.360	0.521	0.344	0.426	§	§	*
Leaf weight (g)	15.4	18.7	34.2	22.8	24.9	§	–	–
Stem weight (g)	1.2	2.0	4.4	2.4	2.6	§	–	–
LAR ($cm^2\,g^{-1}$)	164.1	165.3	145.3	147	170	–	†	–
SLA ($m^2\,kg^{-1}$)	17.8	18.5	17.2	16.1	19.6	–	§	–
Total	17.0	23.1	38.9	25.2	27.5	§	–	–
Jimson weed								
Leaf area (m^2)	0.198	0.219	0.229	0.196	0.232	*	†	–
Leaf weight (g)	11.6	15.8	19.0	14.9	16.0	§	–	–
Stem weight (g)	9.1	15.4	19.3	14.1	15.2	§	–	–
Floral weight (g)	6.1	9.2	9.5	5.8	11.0	†	§	–
LAR ($cm^2\,g^{-1}$)	75.8	57.8	48.9	61.1	60.7	§	–	–
SLA ($m^2\,kg^{-1}$)	17.4	14.4	12.7	14.0	15.6	§	‡	–
Total	27.9	40.4	47.8	34.8	42.2	§	‡	–

*$P<0.10$; †$P<0.05$; ‡$P<0.01$; §$P<0.001$.

LAR, leaf area ratio, the ratio of leaf area by above ground dry weight; SLA, specific leaf weight; Total, total above ground biomass (dry weight). Values are per plant.

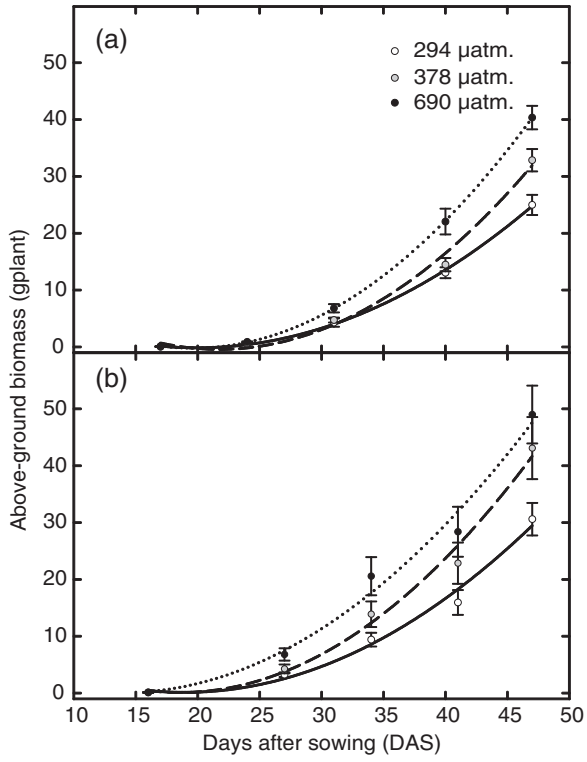

Fig. 2 Same as Fig. 1, but for jimson weed (*Datura stramonium*, g per plant).

tion was reduced, it is important to note that the stimulatory effect of $[CO_2]$ on plant (leaf) growth was such that the overall production of nicotine actually increased significantly for $[CO_2]$ above the 294 μatm early 20th century baseline for both growth temperatures (Fig. 5).

Leaves, as well as all other parts of jimson weed, are recognized as sources of both atropine and scopolamine. In contrast to nicotine, $[CO_2]$ either had no effect, or significantly increased the concentration of atropine and scopolamine, respectively (Table 2). Growth temperature had contrasting effects as well, with significant increases noted for atropine, but not scopolamine (Table 2), although higher concentrations are noted with increased temperature at 26 DAS (Figs 6 and 7). As with nicotine, the stimulatory effect of $[CO_2]$ compensates for any concentration changes at the leaf level so that whole plant production of these secondary compounds significantly increases in response to $[CO_2]$ (Table 2).

Discussion

Production of secondary compounds in plant physiology (i.e. those not involved in primary metabolism) has long been recognized as having a defensive function

(Waller & Nowacki, 1978). Among these secondary compounds, the alkaloids (e.g. atropine, caffeine, cocaine, nicotine, scopolamine) are generally recognized for their role in reducing rates of herbivory by insects or mammals. As such, they play an important role in survivability as well as species success and insect–plant coevolution (e.g. Cheeke, 1989).

However, while the poisonous nature of these compounds is well-known, their usage, depending on dosage, has also been recognized since ancient times as having pharmaceutical/therapeutic value in human systems on a global scale (see Schultes & Reis, 1995 for an overview). In the current experiment, for example,

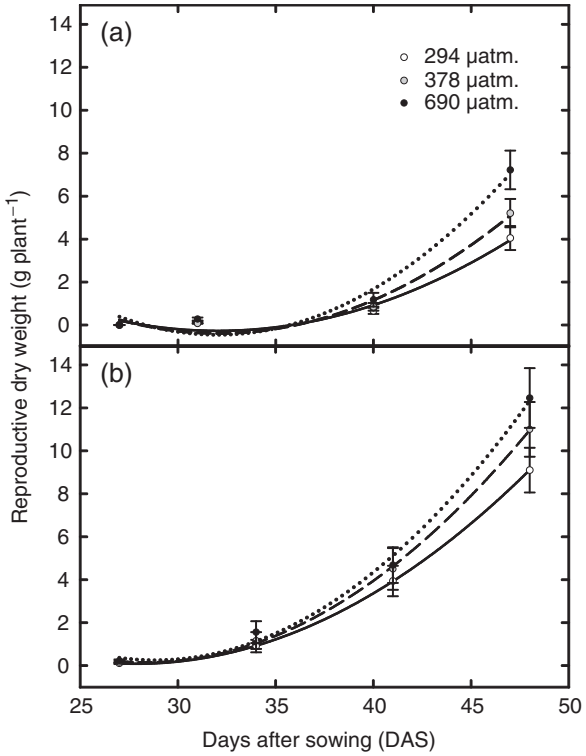

Fig. 3 As for Fig. 2, but for reproductive dry weight of jimson weed. Significant differences in floral weight were observed as a function of [CO_2] by 50 days after sowing (DAS).

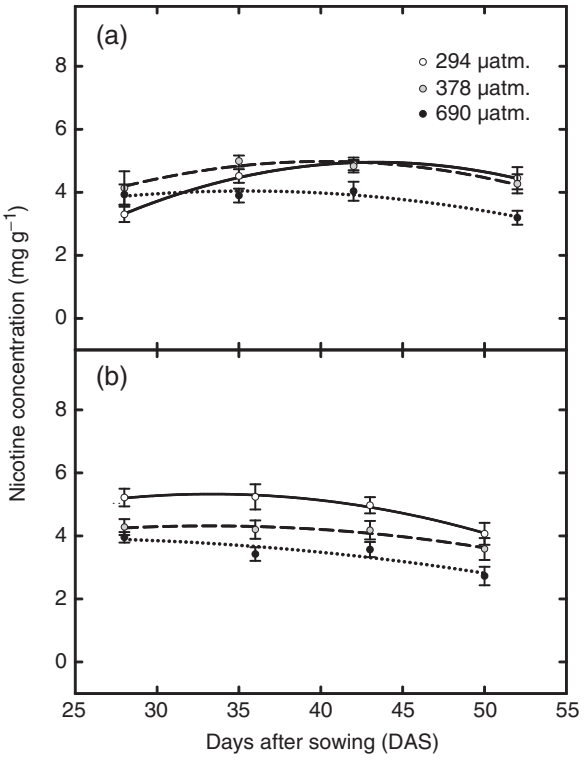

Fig. 4 Leaf nicotine concentration of tobacco (mg g^{-1}) over time at different [CO_2] (294, 378 and 690 µatm) and air temperature (a and b, 22.1, 27.1 °C, respectively). A significant temperature × [CO_2] interaction was observed. Bars are ± SE.

Table 2 Averages and statistical *P* values of the two-way analysis of variance for CO_2 concentration (µatm) and air temperature (°C) effects on secondary products nicotine (tobacco), atropine (jimson weed) and scopolamine (jimson weed)

	Averages					*P*-values		
Variable	294	378	690	22.1	27.1	CO_2 effect	Temperature effect	$CO_2 \times T$
Tobacco								
Nicotine (mg g^{-1})	4.7	4.4	3.6	4.2	4.1	§	–	‡
Nicotine (mg)	31.4	36.4	50.7	38.5	40.6	§	–	–
Jimson weed								
Atropine (mg g^{-1})	1.4	1.3	1.4	1.1	1.7	–	§	*
Atropine (mg)	11.9	14.7	18.9	11.5	18.4	†	‡	*
Scopolamine (mg g^{-1})	1.9	2.2	2.4	2.2	2.1	§	–	–
Scopolamine (mg)	12.9	20.9	25.4	18.8	20.8	‡	–	–

*$P < 0.10$; †$P < 0.05$; ‡$P < 0.01$; §$P < 0.001$.

Data are for all sampling dates (Tobacco) or at 26 and 47 DAS (jimson weed). Data are on a concentration or per plant basis. Per plant values are based on leaf material. Additional details are given in methods.

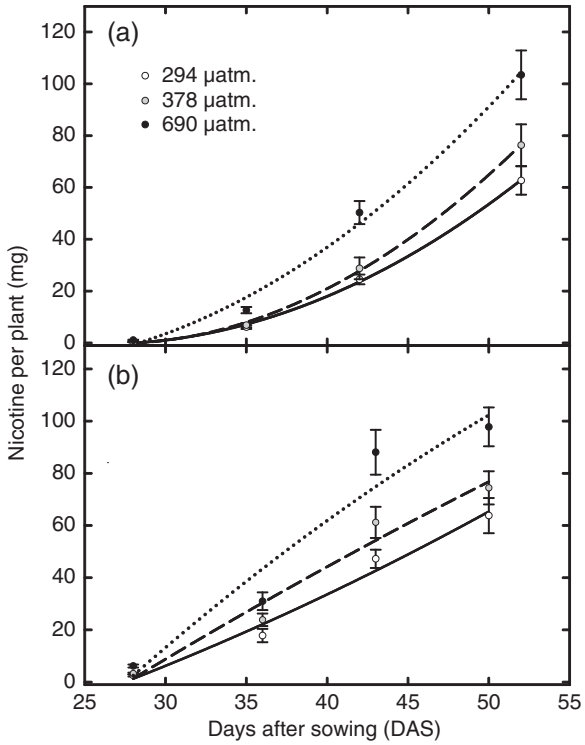

Fig. 5 Whole plant production of nicotine (mg per plant, as determined by leaf biomass and concentration) over time at different [CO_2] (294, 378 and 690 µatm) and air temperature (a and b, 22.1, 27.1 °C, respectively). Bars are ± SE.

the bicyclic alkaloids, atropine and scopolamine, derived from jimson weed (or other members of the belladonna family) are known to be strong anticholinergics. That is, they act as antagonists to acetylcholine at peripheral and central muscarinic receptors, interfering with the transmission of nerve impulses by acetylcholine in the parasympathetic nervous system and producing symptoms typical of parasympathetic system depression: dilated pupils, rapid heartbeat, and dry skin, mouth and respiratory passages. The therapeutic uses of atropine are multiple and include: dilation of pupils in ophthalmology; stimulation of heart rate in patients suffering from bradycardia; and as an antidote to G and V type nerve agents (e.g. soldiers on active duty are given atropine). Because scopolamine depresses the central nervous system, it is also used as a sedative prior to anesthesia and as an antispasmodic in certain disorders characterized by restlessness and agitation, (e.g., delirium tremens, psychosis, mania and Parkinsonism). It is also commonly prescribed in transdermal patches to prevent motion sickness. Conversely, the impact of tobacco-derived nicotine on human health is perceived almost entirely as negative; the addictive qualities of nicotine and its damaging effect on human health being universally acknowledged. Here again however, there are recognized therapeutic effects of nicotine (i.e. unrelated to smoking) that include its use in replacement therapy for tobacco cessation, ulcerative colitis and potential therapy in Parkinson's, Alzheimer's or other cognitive disorders (Westman *et al*., 1995; Rezvani & Levin, 2001, 2002; Yang *et al*., 2003). Although the line between toxicity and benefit is a fine one, the impact of all three alkaloids on human health and well-being is significant and wide-spread.

Overall, in evaluating the role of secondary compounds it is clear that alterations in their production or concentration will significantly affect both evolutionary success (e.g. herbivory), but also their use and efficacy in human systems. In the later instance, it can be argued that synthetic production of these secondary compounds alleviates any concern regarding environmental impacts on their production from botanical sources; however, developing countries (i.e. ~ 75% of the world population) continue to rely on ethno-botanical remedies as their primary medicine (e.g. use of alkaloids from jimson weed as treatment for asthma among native Americans and in India, Stuart, 2004). Furthermore, for both developed and developing countries, there are a number of economically important pharmaceuticals derived solely from plants (e.g. tobacco), whose economic value is considerable (see Table 2, Raskin *et al*., 2002).

It is generally recognized that various aspects of climate such as temperature, soil etc., can alter the production and concentration of secondary compounds (Waller & Nowacki, 1978; Topliss *et al*., 2002). However, alterations in concentration and/or production in response to recent and projected changes in the two principle abiotic aspects of global climate, temperature and [CO_2], have not been well elucidated, particularly with respect to alkaloids of ethno-pharmacological interest.

Yet, it is clear from the current study that [CO_2] and/or temperature may alter either the concentration or production of these alkaloids. At the concentration level, such changes are likely to either increase or decrease rates of herbivory (e.g. tobacco and jimson weed, respectively, in the current study). Previous work has suggested that higher C : N ratios associated with increasing [CO_2] may result in compensatory increases in foliar consumption rates by insects (e.g. Fajer *et al*., 1989). These increased consumption rates are often accompanied (but not always, e.g. Watt *et al*., 1996) by a decrease in the efficiency of plant tissue conversion to body mass, reduced larval growth rate and sometimes reduced pupal mass. While the impact of [CO_2] and/or temperature on concentration is variable, on a per plant basis, increasing [CO_2] above early 20th century levels

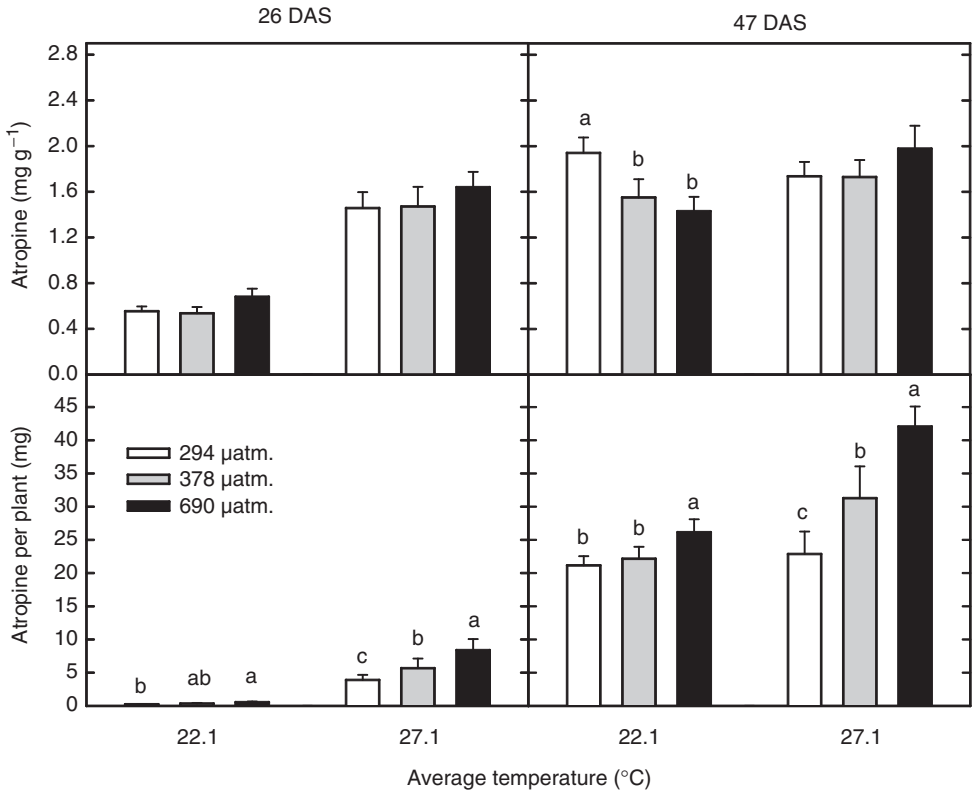

Fig. 6 Concentration (mg g^{-1}, upper graphs) and whole plant production (based on leaf biomass and concentration, lower graphs) for atropine at 26 and 47 days after sowing (DAS) in jimsonweed at different [CO_2] (294, 378 and 690 µatm) and air temperature (22.1, 27.1 °C). Letters indicate Fisher's protected least significant difference differences as a function of [CO_2] for a given air temperature. Bars are ± SE.

resulted in an increase in the total production of all alkaloids examined in the current investigation. This is because the stimulatory effect of [CO_2] on growth compensated for any reduction in concentration (e.g. tobacco). Given the subtle distinctions that exist between toxicity and benefit, [CO_2]/temperature induced changes in their production or ratio (i.e. atropine : scopolamine) will almost certainly influence their efficacy and use in human systems, particularly in developing countries (e.g. jimson weed in Turkey, Uzun *et al*., 2004).

How does rising [CO_2] and/or temperature alter the concentration of these compounds? Nicotine is synthesized from ornithine in the root and transported to the leaves in the xylem (Baldwin *et al*., 1997). Hypothetically, therefore, the direct effect of rising [CO_2] on stomata closure and reduced transpiration could limit nicotine translocation. However, in the current study, increasing temperature, which should increase transpiration, had no effect on nicotine concentration.

Alternatively, the carbon/nutrient balance hypothesis (Bryant *et al*., 1983), predicts that the increase in the C : N ratio of plants exposed to increasing [CO_2] will lower the concentration of nitrogen-based secondary compounds; in part, because increased vegetative growth will require a larger investment of available nitrogen. This hypothesis is consistent with the results observed here for nicotine, which has a lower C : N ratio than atropine or scopolamine. However, previous inquiries regarding this hypothesis in relation to glucosinolate content and [CO_2] have suggested a species specific response (Karowe *et al*., 1997), and it is unclear if [CO_2] will only affect production of those compounds above a critical C : N threshold.

Given that these plants were grown under optimal conditions in the current study, it can be argued that limitations of nutrients or water would negate their response to atmospheric CO_2 *in situ*. However, both tobacco and jimson weed (and many other plants of pharmaceutical interest) are associated with managed environments where water and nutrients may not be lacking (see Patterson, 1995). Still, *in situ* assessments relative to recent changes in atmospheric [CO_2] are difficult because methodological considerations prevent exposure of plants to subambient CO_2 for 24 h periods in the field (see Mayeux *et al*. 1993). This does

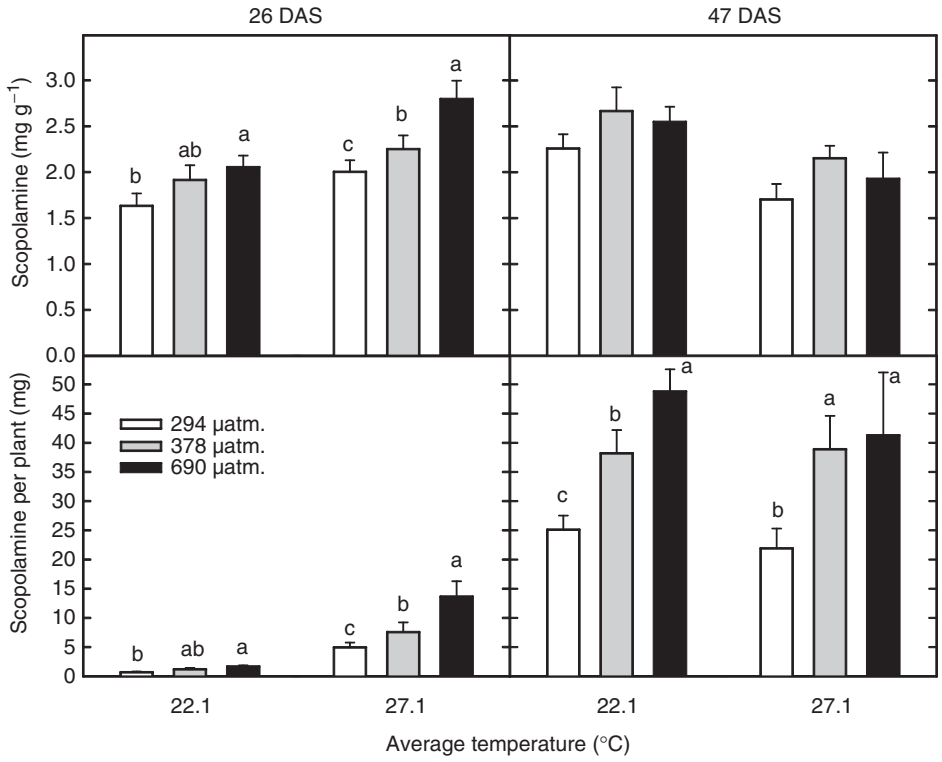

Fig. 7 As for Fig. 6, but for scopolamine.

not, of course lessen the importance of understanding how recent (or projected) increases in atmospheric [CO_2] may have already altered alkaloid concentration.

This is the first study to address alterations in the concentration and production of alkaloids in response to those abiotic factors ([CO_2], temperature) likely to increase concurrently with current and projected anthropogenic changes in global climate. From an evolutionary perspective, changes in secondary metabolite concentration have occurred in the past (i.e. plants evolved at a time when the atmospheric [CO_2] was much higher than today), and herbivore/plant interactions are in a constant state of adjustment as climate varies. However, from an anthropogenic viewpoint, the current increase in these abiotic variables is the highest experienced by human kind since the onset of agriculture (Sage 1995), and it is unclear if the efficacy of ethno-pharmacology has adjusted accordingly.

We recognize that the pharmaceutical role of increasing [CO_2]/temperature in the recent past or projected future cannot be fully elucidated by a single study. Clearly, the responses of the species considered here will vary *in situ* as a function of competition and environment. Nevertheless, understanding the role of climate, particularly the sudden and dramatic rise in atmospheric carbon dioxide within recent decades, as a possible overlooked factor in altering the concentration or productivity of these secondary compounds deserves particular attention. Such an assessment may be especially crucial in determining the use and efficacy of ethno-pharmacological remedies for human sickness in the developing world as atmospheric [CO_2] and global temperatures continue to increase.

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